AGRICULTURAL PLANT BIOCHEMISTRY

Panel Manager - Dr. David Oliver, Iowa State University
Program Director - Dr. Gail McLean

The new Agricultural Plant Biochemistry Program resulted from a need perceived by both the NRI and the plant biology research community for funding for a broad range of plant biochemistry research. The program supports (1) research on biochemical processes in plants and plant symbionts or (2) research using predominantly a biochemical approach to address a problem in plant or plant-microbial biology. Areas of research encouraged by the program include: (1) characterization of primary and secondary metabolic pathways, (2) enzyme reaction mechanisms, (3) structure-function relationships, (4) biochemical compartmentation and interaction between compartments, (5) bioenergetics such as primary electron transfer reactions in photosynthesis and respiration, and (6) assimilation, transport, partitioning, and accumulation of carbon, nitrogen, and other inorganic nutrients.

2000-03429 Enzymatic Mechanism of the Ethylene-forming Enzyme in Plants
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Postdoctoral Fellowship; Grant 2001-35318-10096; $89,800; 2 Years

1-Aminocyclopropane-1-carboxylate oxidase (ACCO) catalyzes the last step in the ethylene biosynthetic pathway in plants. This reaction involves the oxidation of a small molecule, aminocyclopropane carboxylic acid (ACC), to ethylene, with concomitant reduction of molecular oxygen to two water molecules. Ethylene, one of the first chemical messengers to be recognized in plants, signals a wide variety of biologically important processes to occur, such as wilting of leaves, seed germination, and fruit ripening. Over-ripening is a source of enormous loss of agricultural products; thus, the ability to control ethylene production would be of tremendous value. Although the ability of plants to make ethylene, and the consequences of ethylene production, have been known for quite some time, the gene for ACCO was cloned only in the early 1990s. ACCO belongs to a rapidly growing class of enzymes that use a single non-heme iron center to activate molecular oxygen for reaction with organic substrates; a typically forbidden, and thus sluggish, chemical reaction. Many of these non-heme iron enzymes are involved in reactions in metabolic pathways that are environmentally, medically, and agriculturally significant. Unlike heme-containing iron enzymes, these single iron centers do not exhibit the wealth of spectroscopic signals and probes, which makes studying the details of their mechanism difficult. The goals of this proposal are to understand the strategy and chemical mechanism that ACCO uses to activate molecular oxygen for reaction with its substrate in hope of gaining critical information that would eventually aid in the future development of ways to control ethylene production.

2000-03352 Biochemical Genomics of Glucosinolate Biosynthesis in Arabidopsis thaliana
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Grant 2001-35318-10095; $213,000; 2 Years

Glucosinolates are a diverse class of plant-specific secondary metabolites synthesized by cruciferous plants. Although the functions of glucosinolates are unknown, their breakdown products have a variety of biological activities in plants, animals, and humans, which range from plant defense to cancer prevention. Progress has been made in understanding glucosinolate biosynthesis, however, many reactions are hypothetical and most of the genes involved remain to be identified. Using *Arabidopsis thaliana*, a small crucifer and model plant, we propose genomics-based strategies to identifying such genes. We propose synergistic combination of (i) glucosinolate profiling, (ii) gene expression profiling, and (iii) bioinformatics to identify candidate genes. We will catalog tissue-specific glucosinolate profiles during *Arabidopsis* development as well as in response to a large array of environmental cues and chemical agents. Correlation of glucosinolate accumulation with expression of pathway-related marker genes will assist in selecting tissues and conditions for global gene expression profiling. Cluster analysis of gene expression in diverse situations shown to cause differential glucosinolate accumulation is proposed to recognize a common set of co-expressed genes, including genes functionally related to glucosinolate biosynthesis. Based on knowledge of the biochemical pathway, bioinformatics will assist in identifying a subset of potential pathway-related genes. We will test genetically and biochemically the function of candidate genes. Availability of functionally characterized genes will provide molecular tools to favorably manipulate glucosinolate biosynthesis: to reduce antinutritional glucosinolates in *Brassica* seeds for animal feed, and, more importantly, to fortify edible plants with anticarcinogenic glucosinolates for human food.

2000-03449 Pathway and Regulation of C7-Sugar Metabolism in Avocado

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Grant 2001-35318-10001; $150,000; 2 Years; 2000 Award $75,000

The avocado *(Persea americana* Mill) is an important world fruit crop and the United States is the second largest producer worldwide. In California, the avocado industry is under economic pressure to maximize the productivity potential while minimizing the inputs into the agricultural system. The first step towards this goal is to develop a complete understanding of how the tree utilizes and partitions the stored energy resulting from the process of photosynthesis since this will determine the relative rates of fruit and vegetative growth of the tree. To date, our understanding of the processes controlling fruit yield in avocado is very poor. Our work suggests that the photosynthetic processes which produce the energy for the tree, driving productivity, are unique in avocado from several perspectives: (1) avocado utilizes seven carbon (C7) sugars and sugar alcohols as primary forms of fixed carbon, (2) these sugars are transported in the phloem, (3) these sugars will accumulate to high levels in various plant parts involved in energy storage, and finally (4) the fruit will also accumulate these sugars and do not ripen unless and until these sugars are metabolized. This project will examine the physiology and biochemistry of these C7 sugars in avocado with the goal of understanding the processes governing the synthesis of C7 sugars in leaves, their movement throughout the plant, and their role in fruit ripening. This information will be particularly useful in

expanding our knowledge of the postharvest biology of avocado and the factors governing fruit ripening.

2000-01145 Proteomic Analysis of Symbiosome Proteins from Medicago Root Nodules

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Seed Grant 2001-35311-10161; $46,800; 1 Year

The aim of our work is to understand the molecular mechanisms underpinning root nodule formation and function. Nitrogen fixing root nodules are small growths that are formed on plant roots by the naturally-occurring soil bacteria rhizobia. Within these beneficial nodules, atmospheric nitrogen is reduced into a form that can be utilized by the plant, and this form of nitrogen assimilation greatly minimizes the amount of chemical fertilizers required for productive crop growth. Therefore, this natural type of nitrogen uptake can protect the environment from potential contamination by chemical fertilizers and also reduce the farmer's cost of crop production. Important crop plants such as soybean, pea and peanut can take advantage of this type of nutrient acquisition.

To understand how nodules are formed and how they function, we have established a program to study the protein composition of the symbiosome membrane. The symbiosome membrane is a unique structure that surrounds the bacteria within the root nodule. The symbiosome membrane plays a critical role in communication between the host and microbe and for the exchanges of compounds between the two organisms, allowing a "successful' interaction to occur. We are using a powerful biochemical method called proteomics to identify and study proteins from the symbiosome membrane. The results of our work can be applied to plant breeding efforts to produce plants with the increased capacity to form nitrogen-fixing root nodules.

2000-03356 Manipulation of starch debranching enzyme activities in transgenic plants

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Grant 2001-35318-10003; $150,000; 2 Years; 2000 Award $75,000

Starch is an important agricultural commodity and renewable raw material. Starch utility stems largely from the chemical and structural features of the glucose polymers that constitute starch granules. Modification of starch structure by alteration of chain lengths or branch frequency has provided starch forms with different functional properties, thus broadening starch usage. Further expansion of starch utilization will depend on a thorough understanding of the molecular mechanisms that determine starch structure in the plant, as well as the ability to manipulate specific aspects of that enzymatic machinery. Previous genetic analyses indicate that starch debranching enzymes play an important role in synthesizing starch, but their precise functions are not clear. This research aims to test the hypothesis that debranching enzymes provide critical editing functions that help determine the distribution of chain lengths and branch linkages in starch, by manipulating debranching enzyme expression in transgenic maize and potato

plants. Transgenic plants will be generated that either increase or decrease the expression of two types of starch debranching enzymes, termed isoamylase-type and pullulanase-type enzymes. The structures of the starches that accumulate in kernels or tubers of the transgenic plants will be characterized in detail, and correlated with the expression level of each type of debranching enzyme. This approach offers a unique opportunity to modulate enzyme activities within plants, and to investigate the specific relationship between starch structure and starch debranching enzyme function. In addition, this research may lead to the formation of novel starch structures with potential utility for industrial applications.

2000-03447 How is acetyl-CoA generated in plants?

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Grant 2001-35318-10094; $225,000; 3 Years

Acetyl-CoA is a precursor of a variety of phytochemicals that are crucial to plant growth and development (*e.g.*, membranes, lipid signal molecules, brassinosteroids, flavonoids). In addition, many of these compounds are important agricultural products (*e.g.*, oils, natural rubber, fragrances, and essential oils). Despite the importance of acetyl-CoA, and its central position in carbon metabolism, its generation for the biosynthetic processes is poorly understood in plants. Our previous research indicates that plants may have a complex set of mechanisms for generating at least two distinct acetyl-CoA-pools (cytosolic and plastidic) that are each a precursor for the biosynthesis of different sets of phytochemicals. We have isolated the genic sequences of five enzymes that we hypothesize are involved in generating these distinct acetyl-CoA pools. These enzymes are pyruvate decarboxylase (PDC), acetaldehyde dehydrogenase (ALDH), acetyl-CoA synthetase (ACS), plastidic pyruvate dehydrogenase (ptPDH), and ATP-citrate lyase (ACL). Using these genic sequences, we are developing molecular reagents (DNA probes and antibodies) for detecting each of these gene products. In addition, we are developing genetic stocks (transgenic plants) that show perturbed expression of each of these genes in leaves and roots. Using our collection of molecular reagents we will determine the role of each enzyme (and the corresponding genes) in generating the two distinct pools of acetyl-CoA in leaves and roots. These data will provide insights into the physiological regulatory mechanisms that control the flow of carbon among highly networked metabolic processes. Such fundamental understanding will lay the foundation for the rational improvement of the agricultural production of a variety of acetyl-CoA-derived phytochemicals.

2000-03357 Symposium on Biosynthesis of Glucose Polysaccharides

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Grant 2001-35318-10123; $10,000; 1 Year

The most abundant type of chemical compound that occurs in living organisms is the glucose polymer, examples of which are glucose, starch, and glycogen. The biosynthesis of these compounds is under intensive study worldwide although individual

laboratories typically focus on one type of polymer without considering the instructive similarities and differences in the related biosynthetic systems. To aid the research community, this project will provide the funds to make possible a conference entitled "Biosynthesis of Glucose Polysaccharides," to be held at Iowa State University from June 29 to July 2, 2000. The goal of this conference is to engender thought and discussion between research groups working on starch, glycogen, or cellulose biosynthesis.

2000-03453 Structure and function of VAR2, a plastic charonin with homology to E. coli FtsH

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Grant 2001-35318-10004; $225,000; 3 Years

Chaperones are proteins that assist in the proper folding, refolding or assembly of other proteins. Some ATP-dependent proteases, termed "charonins," have intrinsic chaperone activity and provide a quality control mechanism to rid the cell of misfolded, partially-folded or unassembled proteins. FtsH is one of the best-known charonins in prokaryotes and eukaryotes, but our knowledge of FtsH structure and function is not well understood. We have recently cloned the VAR2 gene in Arabidopsis and found that it encodes an FtsH homolog; mutant var2 plants have a variegation phenotype due to a failure of chloroplasts to develop normally in the white sectors. In this proposal we will examine the function of VAR2 and determine those structural elements of the protein that are important for function. We will examine, first, the temporal and spatial patterns of VAR:2 expression. Second, we will identify proteins that interact with VAR2 (*i.e.*, substrates and proteins that modulate VAR2 activity) using interaction cloning and by characterizing second-site suppressors of var2. Third, insight into structure/function relationships will come from the characterization of an allelic series of var2 and by the generation and characterization of FtsH mutants of the cyanobacterium, Synechocystis 6803. Finally, we will gain insight into the mechanism of var2 variegation by second site suppressor analysis and by studying transgenic Arabidopsis with altered amounts of VAR2 protein (antisense and over-expression plants).

2000-03367 Carboxyl methylation of isoprenylated proteins in Arabidopsis

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Grant 2001-35318-10091; $225,000; 3 Years

Drought can cause billions of dollars in crop loss, with an even greater impact on the overall economy. It is, therefore, important to develop better strategies for preventing crop loss due to drought. Recently, it was discovered that plants incapable of performing a specific type of protein modification are drought tolerant. Many proteins in plant cells are modified by the addition of an isoprenoid tail and a terminal methyl group. Mutations that abolish the gene responsible for the addition of the isoprenoid tail cause plants to exhibit an enhanced response to ABA, which is an endogenous hormone necessary for normal water relations. Consequently, the stomates of these plants, which are small apertures involved in gas exchange, are closed and the plants lose less water under drought conditions. Since the addition of the methyl group is equally important for the

function of these modified (*i.e.*, isoprenylated) proteins, it follows that plants engineered to be incapable of performing the methylation reaction will also exhibit drought tolerance.

We have recently cloned the gene that encodes the enzyme responsible for methylation of isoprenylated proteins. The function of this gene has been confirmed and we are now developing genetically modified plants that lack the ability to methylate isoprenylated proteins. Understanding how these plants behave under drought conditions will not only provide a better understanding of environmental adaptability in plants but may also lead to the development of drought tolerant crops.

2000-03459 Function of Plant Hexokinase as a Sugar Sensor

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Grant 2001-35318-10002; $225,000; 3 Years

Plant sugars are known to function not only as metabolic fuels and substrates, but also as signal compounds that affect the expression of many different genes involved in seed germination, root and shoot growth, photosynthesis, flowering, and senescence. The long-term goal of this research is to understand sugar sensing mechanisms by examining the function of sugar sensors, by identifying the components of the signal transduction processes, and by evaluating sugar-dependent modulation of target processes that ultimately control plant growth. The best understood plant sugar sensor/transducer is the dual-function protein hexokinase (HXK). This protein also has the metabolic role of controlling the entry of glucose into primary metabolism, by forming glucose 6-phosphate. Preliminary data indicate that in the model organism, *Arabidopsis*, HXK protein can be structurally manipulated to eliminate glucose phosphorylation without impacting glucose signaling. Preliminary data also indicate that HXK protein occurs in a large complex with vacuolar ATPase and other unidentified proteins, and that HXK protein has an unusual subcellular association with a variety of endomembranes. The proposed research has two objectives: (1) Identify the structural determinants of HXK protein required for glucose signaling; and, (2) Identify proteins in the HXK complex and their link to sugar signaling. These experiments will greatly define the roles of protein-protein interactions, subcellular compartmentation, and protein phosphorylation as components of HXK-dependent glucose signal transduction.

2000-03393 Function of Glucose-6-Phosphate Dehydrogenase in Developing Oil Seeds

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Grant 2001-35318-10090; $225,000; 3 Years

Seeds and their storage compounds, *e.g.* oil, often represent the highest value of a crop plant. In the future, renewable resources become increasingly more important as replacement for mineral oil. Therefore, it is highly desirable to increase seed oil yield. Genetic engineering techniques enable us now to directly accomplish this goal. Typically, genes are targeted that contain the blue print for highly regulated enzymes. As we are entering the post-genome era, <u>all</u> potential target genes for genetic engineering

will become available. Fatty acids are the main constituents of seed oil. Their biosynthesis requires not only large amounts of two-carbon units, but even larger amounts of chemical reducing power. It is postulated that the availability of this reducing power may be limiting to oil biosynthesis. In developing seeds, a specific glucose oxidation pathway with its key enzyme glucose-6-phosphate dehydrogenase is a likely source for reducing power. The role of this pathway and its key enzyme in seed fatty acid biosynthesis will be investigated in Arabidopsis, the first plant for which the entire genome is known. Genomic analysis will be used to identify genes encoding seed glucose-6-phosphate dehydrogenases. The construction of transgenic plants will permit a functional analysis of these enzymes. Overexpression of a bacterial gene encoding a deregulated glucose-6-phosphate dehydrogenase should override the regulatory properties of the respective plant enzymes. The transgenic plants should reveal whether reducing power may be limiting to oil biosynthesis. The results may suggest new avenues towards increasing seed oil yield.

2000-03496 Characterization of Allantoinase

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Grant 2001-35318-10005; $180,000; 3 Years

We propose to characterize the biochemical reactions by which plants and microorganisms use two naturally-occurring, nitrogen-containing compounds: allantoin and urea. Allantoin is produced in many plants, including soybeans, through the action of symbiotic bacteria that "fix" nitrogen gas in special organelles found in the roots. The plants use the fixed nitrogen to synthesize allantoin and then transport this compound from their roots throughout their tissues. The plants transform allantoin to a more useable nitrogen-containing nutrient by action of the enzyme allantoinase. We plan to purify and characterize this poorly studied enzyme. In particular, we plan to test our hypothesis that allantoinase possesses a metal-containing catalytic site that is similar to one present in urease. At the same time, we will continue our efforts to better characterize urease, an enzyme for which we have determined the three-dimensional structure. Urease is used by plants to transform urea into ammonia, a more useful form of nitrogen for growth. Urea is both synthesized by plants and provided to crops in the form of urea-based fertilizers. Plant growth often is limited by a lack of available nitrogen; thus, our studies of allantoinase and urease are of fundamental interest to agriculture and may lead to long-term improvements in crop yield.

2000-03497 Phenylpropene Flavor Compounds in Basil: Biosynthesis and regulation

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Grant 2001-35318-10006; $180,000; 3 Years; 2000 Award $65,000

Plants produce chemicals in their leaves to protect themselves against herbivores. Humans have found many such chemicals attractive as flavor and scent compounds (*i.e.*, spices), and have also used them to protect their foods from spoilage by microorganisms and insect pests. One class of plant defense compounds that have been used extensively as spices by humans are the phenylpropenes, which include eugenol (the spice of clove).

Plants that contain high concentrations of these compounds have high economic value. Our understanding of the biosynthetic pathway that produces these compounds in the plant, however, has remained incomplete. To elucidate the biochemical pathway that leads to the formation of the phenylpropene flavor compounds, we will use basil (*Ocimum basilicum*) as the model system. We will identify the unknown intermediates in the phenylpropene pathway using labelled precursors, and we will purify and characterize the enzymes that catalyze their formation. We will also isolate and characterize the genes encoding these enzymes. Finally, we will examine the molecular and biochemical mechanisms by which basil leaves control the synthesis of these defense/flavor compounds, with a view toward manipulating the production of these compounds in basil and genetically engineering this pathway into new plant crops for added value and disease resistance.

2000-03364 Soybean Urate Oxidase and the Ureide Pathway
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Grant 2001-35318-10097; $225,000; 2 Years

Nitrogen is a limiting nutrient for most crops; legumes such as soybeans occupy a special place in agriculture because they possess the ability to convert atmospheric nitrogen into ammonia. The metabolic pathway by which the ammonia is converted into organic compounds that can be used by the plant to support its growth is called the ureide pathway. Nitrogen fixation is an energetically costly process for the plant, and a worthwhile goal for agricultural research is to enhance the efficiency of the process by which nitrogen becomes available to the plant in usable form. A prerequisite for doing so, however, is gaining a detailed understanding of the ureide pathway and its component enzymes. One of the enzymes in the ureide pathway is urate oxidase, which catalyzes the conversion of urate to 5-hydroxyisourate. It is not known how 5-hydroxyisourate is converted to allantoin, which is the metabolite that the plants transport from the roots, where nitrogen fixation and the urate oxidase reaction occur, to the stem and leaves, where the nitrogen is used in amino acid biosynthesis.

One current goal is to characterize 5-hydroxyisourate hydrolase, which may be a hitherto unrecognized constituent of the ureide pathway. A second goal is to continue mechanistic studies of urate oxidase by isolating and identifying intermediates formed during the catalytic cycle. These studies are leading to an understanding of how urate oxidase can operate in the absence of any of the cofactors that typically mediate dioxygen-dependent enzymatic reactions.

2000-03348 Homeostasis of Manganese in a Photosynthetic Organism
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Grant 2001-35318-10007; $180,000; 3 Yyears; 2000 Award $70,000

The goal of this project is to define how transport of elemental manganese takes place across the cellular membranes of the photosynthetic organism *Synechocystis* sp. PCC 6803, a cyanobacterium. The transition metal manganese (Mn) is an essential element for all organisms. In particular, it plays a critical role in the life-cycle of plants and other photosynthetic organisms since their ability to generate oxygen depends on a

manganese cluster in the photosystem II complex. We have identified an ABC transporter complex for Mn in the plasma membranes of *Synechocystis 6803,* the first of its kind in any organism. This bacterial permease, MntABC, has three essential protein components: (a) MntA, a cytoplasmic protein with ATP binding motifs, (B) MntB, an integral membrane protein, and (c) MntC, a periplasmic Mn-binding protein. Our previous studies have also established the presence of a second high affinity transporter for manganese in addition to the MntABC complex in these cyanobacterial cells. We will use mutagenesis of amino acids followed by biochemical analysis to determine regions of this protein complex that are important for the binding and transport of Mn. We will also undertake genetic studies to identify the gene(s) and protein(s) of the second transporter and analyze its properties. Together, these aims should result in a better understanding of the transport of manganese in this photosynthetic organism. The information from these studies can also be applied to the understanding of manganese transport mechanisms in plants and animals.

2000-03395 Enhancement of Wheat Sink Strength and Yield

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Grant 2001-35318-10093; $150,000; 2 Years; 2000 Award $75,000

The yield of cereal crops is intimately linked to plant processes involving both the production of sugars in photosynthesis and the storage of photosynthate in storage organs such as seeds. In cereals, the starchy endosperm is the predominant sink into which photosynthate is funneled. The fixed maximal rate of starch biosynthesis in the endosperm of cereals likely exerts limitations on both plant growth and yield. These limitations are the result of low levels of important starch biosynthetic enzyme activity. The low activity levels may be due to either low inherent amounts of these enzymes or negative allosteric control of rate limiting enzymes. One such rate limiting enzyme is ADPglucose pyrophosphorylase (AGP), which catalyzes the first committed step in starch biosynthesis and is subject to negative allosteric control by phosphate. An altered subunit of maize AGP has been shown to render the AGP holoenzyme insensitive to inhibition by phosphate and increase seed size in maize by 15%. We are interested in the effects that this de-regulated AGP activity may confer upon wheat. We have created an initial population of transgenic wheat expressing the modified maize AGP subunit. Initial experiments indicate that the transgene is expressed and may modify plant growth and development positively impacting plant yield. This transgenic approach in wheat using the modified AGP subunit is aimed at testing the effects of increased sink strength on production, transport, and accumulation of carbon in cereals. Results may indicate that significant increases in agronomic yield are possible by manipulating seed sink strength.

2000-03422 Activation of 14-3-3 Proteins by Divalent Cations, Protons and Polyamines

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Grant 2001-35318-10185; $225,000; 3 Years

The '14-3-3 proteins' are highly conserved proteins that occur in both plants and animals. In animals, they function in the regulation of a wide variety of enzymes and as components of various signal transduction pathways. In plants, they function in the control of gene expression—as components of trans factor complexes—and in the regulation of several metabolic processes including nitrate assimilation. In all of these cases, the 14-3-3s function as binding proteins; they recognize, and bind to, a relatively simple sequence of amino acids that is found in a variety of proteins. Interestingly, however, an 'activator' such as a divalent cation (e.g., Mg^{2+}) is required for 14-3-3s to bind to other proteins, indicating that their function may be regulated. The divalent cations bind to the 14-3-3 and cause a conformational change that we postulate 'opens' the polypeptide-binding surface. Recent results suggest that protons (e.g., slightly acidic pH such as pH 6.5) or surprisingly dissimilar compounds such as the polyamines (spermine and spermidine) can substitute for the required divalent cation. A major goal of the proposed work is to characterize the role of cations—including protons, divalent cations and polycations—in activating 14-3-3s, and to identify the respective binding sites. We are postulating that divalent cations and polycations bind to the same site on the 14-3-3s, possibly a loop region, whereas the effect of protons may involve histidine residues found elsewhere in the protein. We will test these postulates using directed mutagenesis and by producing truncated mutants that lack certain regions of the protein. Because the 14-3-3s are potentially involved in regulation of metabolic processes that control plant growth and development, it is essential to have fundamental knowledge of these proteins and the roles they play. This fundamental information may provide the novel strategies needed to increase crop yield potential to meet the increasing global demand for food and fiber in the future. This work may also contribute to our understanding of how stress affects plant metabolism, because biotic and abiotic stresses often cause cellular pH to become more acidic and often result in accumulation of polyamines. Understanding the components involved may provide strategies to increase plant tolerance to stress.

2000-03466 11th International Conference on Arabidopsis Research

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Conference Grant; Grant 2001-35318-09906; $10,000; 1 Year

The 11th International Conference on Arabidopsis Research will be held in Madison, Wisconsin June 24 through June 28, 2000. *Arabidopsis thaliana* has been the subject of genetic study for many years. However, during the last decade, the number of research laboratories using Arabidopsis as a model system has increased tremendously, and Arabidopsis is currently being used to study all aspects of plant biology. The rapid rate of progress in Arabidopsis research, including the near completion of the genomic sequence, underscores the usefulness of holding a meeting every year. These conferences provide an important opportunity for the Arabidopsis community to interact and exchange information. The meeting will provide opportunities for both formal and informal exchanges of recent information through platform and poster presentations. The meeting will open with an evening keynote address on the global impact of plant biology to be delivered by Richard Jefferson, the Executive Director of CAMBIA (Center for the Application of Molecular Biology to International Agriculture). This will be followed by

short updates from each of the NSF-funded Plant Genome groups. Many of these groups are carrying out projects that impact the Arabidopsis community. Each of the 17 platform sessions will consist of talks from two invited speakers followed by two short talks that have been chosen from the submitted poster abstracts. A concerted effort has been made to invite junior investigators, including graduate students and postdocs, to give these talks. Posters will be available for viewing during three formal sessions.

2000-01128 Centrifuge for Applications in Plant Phospholipase D Research
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Equipment Grant; Grant 2001-35311-10196; $27,641; 1 Year

The purpose of this proposal is to obtain funding for a centrifuge to be used in research involving plant biochemistry. Specifically this research aims at determining the role that the enzyme Phospholipase D (PLD) plays in the plant. Response to environmental conditions and regulation of growth and development are important processes in all living systems. PLD is involved in how plants respond to harsh environmental conditions like drought and cold stress, in normal growth and development, and in the final stages of plant life, called senescence. So investigations addressing how PLD functions will provide a better understanding of plant growth and development, which has direct potential to long-range improvement in and sustainability of U.S. agriculture.

As in animals, plants are composed of many different tissues, which are in turn made up of specific proteins serving a particular role in the tissue. A common biochemical technique involves homogenization of the tissue in a buffer solution followed by centrifugation, a high-speed spinning of the homogenate. This results in a separation of the various tissue components—some end up in a solid pellet at the bottom of the centrifugation tube, while other components (like the protein enzyme PLD) remain soluble in the buffer solution. Thus the centrifuge provides a separation that is one of the initial, critical steps necessary for characterization of PLD. Along with other faculty research on plant development, this centrifuge will provide student research opportunities in a variety of courses. Matching funds are provided by Montclair State University.

2000-03374 Regulation of [Fe-S] Cluster Biogenesis in Photosystem I
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Grant 2001-35318-10125; $180,000, 3 Years

Photosynthesis provides the metabolic energy by which plants grow and reproduce. The photosystem I and photosystem II reaction centers function to furnish this energy by converting sunlight into oxygen, adenine triphosphoate, and nicotinamide adenine nucleotide phosphate. Both reaction centers contain metal complexes that transfer electrons as a response to excitation by light. One aspect of the work focuses on the genes and proteins that are involved in the biosynthesis of the [4Fe-4S] clusters that comprise the F_X, F_B and F_A electron acceptors in photosystem I. By analogy with the iron-sulfur cluster assembly genes in *Azotobacter vinelandii* and *Escherichia coli*, we

have identified eight genes in the *Synechocystis* sp. PCC 6803 genome that code for cysteine desulfurase, iron mobilization, ferredoxin, and iron-sulfur cluster assembly proteins. The function of these genes will be determined by interruption mutagenesis and by functional assays after the proteins are expressed in *Escherichia coli*. A second aspect of the work focuses on the role of a gene that is involved in the turnover of photosystem I complexes that contain altered iron-sulfur clusters. The protein coded by this gene is able to identify a damaged reaction center and either target it for turnover or participate in the process of proteolysis. The overall goal is to understand the role of genes of as-yet unassigned function in the assembly of photosystem I so as to devise novel strategies to increase the efficiency of photosynthesis in plants. This project therefore has relevance to long range improvement in and sustainability of agriculture.

2000-03463 Photosynthesis Gordon Conference

Brudvig, G.W.

Gordon Research Conferences; P.O. Box 984; West Kingston, RI 02892-0984

Conference Grant; Grant 2001-35318-10092; $10,000; 1 Year

This grant application requests support for a Gordon Research Conference on "Biophysical Aspects of Photosynthesis" to be held at the Kimball Union Academy, NH from June 18 to 23, 2000. The conference Chair is Dr. Gary Brudvig, Department of Chemistry, Yale University and the Vice-Chair is Dr. Donald Bryant, Department of Biochemistry and Molecular Biology, The Pennsylvania State University. Gordon Research Conferences on Photosynthesis are held every two of three years. One of the two conferences is devoted primarily to the biochemical aspects of photosynthesis and the other to the biophysical aspects of photosynthesis. The International Congress on Photosynthesis is held every third year in rotation with the Gordon Research Conferences. The Gordon Research Conferences are key conferences in the photosynthesis field and are attended by researchers from all over the world. The objective for the 2000 Gordon Research Conference on "Biophysical Aspects of Photosynthesis" is to bring together a diverse group of scientists who bring to bear a range of expertise on the problems in photosynthesis. Leading researchers will present lectures and extended time will be devoted to discussion of the latest results. The program for the 2000 Gordon Research Conference on "Biophysical Aspects of Photosynthesis" is posted on the Gordon Research Conference web site (http://www.grc.uri.edu/programs/2000/photo.htm) and it appeared in the February 11, 2000 issue of *Science*.

2000-03359 Support for 'Molecular Basis of Microbial One-Carbon Metabolism' Gordon Research Conference

Arp, D.J.

Gordon Research Conferences; University of Rhode Island; West Kingston, RI 02892-0984

Conference Grant; Grant 2001-35318-10009; $10,000; 1 Year

Funds will provide partial support of an upcoming international conference, the Gordon Research Conference on the "Molecular Basis of Microbial One-Carbon Metabolism". This Gordon Research Conference (GRC) will be held from July 8 to July 13, 1999 at Connecticut College in New London, Connecticut. Prior to becoming

affiliated with the GRC, this conference was held every 3 years in different parts of the world including Japan, Germany, The Former USSR, The Netherlands, The United Kingdom, and Minneapolis (1993) and San Diego (1995) in the U.S. Affiliated with GRC for the first time in 1998, this prestigious conference is now held every two years. The Conference (tenth in the series) brings together the international community of scientists who research aspects of the molecular mechanisms by which microorganisms metabolize and grow at the expense of one-carbon compounds (i.e., carbon monoxide, carbon dioxide, methanol, methane, formate and methylamine). These organisms play important roles in the agriculture and the environment, including interactions with plants. The topics covered vary from basic molecular biology, biochemistry, and genetics to global cycling and bioremediation. The small size and traditional informality of the GRC format provides a unique opportunity for researchers from different disciplines to interact, share information, and develop long-lasting ties.

2000-03360 Gordon Research Conference on Nitrogen Fixation

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Gordon Research Conferences, West Kingston, RI 02892
Conference Grant; Grant 2001-35318-10008; $7000; 1 Year

The fourth Gordon Research Conference on the Biochemistry, Chemistry and Regulation of Nitrogen Fixation will be held at Colby-Sawyer College in New Hampshire from July 2 - July 7, 2000. The opening session of the meeting will include an overview talk on the biochemistry and chemistry of nitrogenase. The goal for this session is to frame the questions to be addressed during the meeting and to provide background given the science diversity of the participants. We have also included a session on related systems, with lectures covering the broad area of metallocenter assembly. The general session will include regulation of nitrogen fixation, two sessions on the structure and mechanism of nitrogenase, a session on the chemistry of nitrogen fixation, a session on the assembly of the metallocenters of nitrogenase, and a session on the nitrogen cycle. Several new features have been integrated into this meeting. More time has been provided for discussions after each talk, and time has been integrated throughout the meeting for more general discussions. The goal is to better foster the open exchange of ideas that can only occur at a meeting such as this. We will have a poster presentation session chaired by a young scientist in the field, with speakers for this session selected from submitted poster abstracts. One of the goals of this session will be to provide an opportunity for younger scientists (especially graduate students and postdoctoral fellows) to present their results at a Gordon Conference.

2000-01228 Enhancing Kentucky Bluegrass Forage Quality through Physiological and Molecular Approaches

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Equipment Grant; Grant 2001-35311-09995; $50,000; 2 Years

Kentucky bluegrass is an important forage grass in the north central and northeastern regions of the United States, but herbage yield and quality improvements are desired, particularly in soils that are low in nitrogen. Nitrogen is the fertilizer element

required in largest amounts for the growth of forage grasses, and application of nitrogen fertilizers can increase forage yield and protein content. However, high levels of nitrogen fertilizers often result in elevated levels of nitrate, a potentially hazardous chemical, in ground water and grass tissues. As the demand for safer food and a healthier environment increases, a molecular approach to enhancing forage quality and quantity with lower input of nitrogen fertilizers must be sought. The ability of Kentucky bluegrass to grow well in low-nitrogen soils may be enhanced by increasing nitrate absorption and use efficiency. Genes such as those encoding nitrate transporters in roots and nitrate reductases - key enzymes in nitrate assimilation - regulate nitrate uptake into and utilization within the grass. Our objective is further understanding of the physiological and genetic mechanisms of efficient nitrate uptake and metabolism in Kentucky bluegrass, with the long-term goal of enhancing its forage quality and quantity with lower input of nitrogen fertilizers. We will study nitrate transporter and nitrate reductase genes and identify a suitable approach to studying tissue nitrate pools. Results from this research will lead to engineering of Kentucky bluegrasses that have better forage quality and support greater animal performance but need no more, or possibly less, nitrogen fertilizer than is currently applied.

2000-03455 A functional genomics approach to studying crop plant triterpenes
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Grant 2001-35318-10157; $225,000; 3 Years

The triterpenes comprise a large and structurally diverse group of natural products. Most of these are nonsteroidal plant compounds, and few of their biological roles have yet been determined. Genomic sequencing efforts have uncovered numerous oxidosqualene cyclases (enzymes that form cyclic triterpene skeletons) in crop plants. These sequences suggest functions for triterpenes in crop plants and offer an avenue to studying their function and biosynthesis. We plan to: (1) Use information from genomic sequencing projects to clone oxidosqualene cyclases from crop plants, and determine what triterpenes they produce. We will express the enzymes in yeast , incubate them in vitro with synthetic oxidosqualene, and determine the structures of their products by NMR spectroscopy. (2) Identify catalytic residues by mutating residues with conservation patterns that correlate with product structure and then determining which compounds the resultant enzymes produce. (3) Use phylogenetic analysis to determine when these enzymes evolved. These experiments will eventually provide a comprehensive accounting of the triterpene skeletons crop plants produce. Recombinant technology should readily provide compounds that these plants produce in amounts insufficient for conventional natural product isolation. Identifying catalytically essential residues for specific triterpene skeletons will allow subsequent identification of newly discovered genes using sequence alone. Characterizing the evolutionary history of each gene will allow us to predict which triterpenes are produced by other plants that have not been sequenced or experimentally examined. This work will lay the groundwork for subsequent efforts to identify gene expression patterns, and to generate mutant plants with altered triterpene levels.

2000-03458 Structural Studies on the Role of MgATP in Nitrogenase Catalysis

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Grant 2001-35318-10122; $180,000; 3 Years

Biological nitrogen fixation is a process by which microorganisms convert atmospheric nitrogen to ammonia. The ammonia generated in this process is utilized by plants as a source of nitrogen for the synthesis of necessary proteins and nucleic acids. Since the industrial production of fertilizers is highly costly and can produce environmentally unfriendly byproducts, research on biological nitrogen fixation is of agronomic, economic, and environmental importance. In all known biological systems, nitrogen fixation is catalyzed by the enzyme nitrogenase. In our research we are interested in the role of MgATP in nitrogenase catalysis. MgATP contains energy in the form of high-energy bonds that drive the overall process and is a general mechanism used to drive numerous cellular processes including cell differentiation, DNA replication, and protein synthesis. During nitrogenase catalysis, the binding and hydrolysis of MgATP confer conformationally distinct structural states on the nitrogenase complex that promote macromolecular complex formation and electron transfer reactions directed at the conversion of nitrogen to ammonia. With the use of MgATP analogs and site-directed variants of the nitrogenase components we are able to trap various conformations of the enzyme and characterize them by X-ray diffraction methods. This supplies us with various snapshots of the nitrogenase at work and from these snapshots we can better understand the fundamental mechanism of nitrogenase catalysis and the manner in which the MgATP binding and hydrolysis are harnessed to drive this fundamental process.

2000-03376 Biochemistry of Signal Termination in Plants
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New Investigator Award; Grant 2001-35318-10124; $180,000; 3 Years

All rganisms require the ability to respond to their environment in order to adapt and survive. Second messengers are molecules that allow individual cells within the organism to respond to signals generated outside of the cell. Second messengers are produced within the cell in response to signals and allow for amplification of the signal. Second messengers appear to accumulate rapidly and transiently in response to signals, and this type of regulation is important for discreet cellular responses and downstream biological effects. My research group has identified eleven genes from the model plant, *Arabidopsis thaliana,* that encode enzymes predicted to hydrolyze the second messenger inositol (1,4,5)-triphosphate (IP3). We predict that these enzymes are capable of controlling IP3 second messenger levels in plant cells and are used to terminate signaling events. This hypothesis will be tested by analyzing various qualities of these enzymes including where in the plant they are made and which second messengers they can hydrolyze. We will also identify mutant Arabidopsis plants that have a loss-of-function of one of these enzymes to determine what biological role these enzymes play. As plants in the field respond to a variety of signals such as drought, pests and pathogens, this work will help identify the molecular machinery plants use to respond to these signals. The

identification of new targets for modulating signal transduction may permit the engineering of transgenic plants with altered physiological responses.

2000-01122 Acquisition of a Fluorescence Spectrophotometer/Luminometer for Plant Research.

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Equipment Grant; Grant 2001-35311-09997; $17,235; 1 Year

All plants depend on the energy of sunlight for growth through a process known as photosynthesis. The proper regulation of photosynthetic reactions during the transitions from daylight to night and back again is critical for maintaining optimal crop yield. The site of the photosynthetic reactions in plants is the chloroplast. Recent evidence suggests that the movement of calcium and magnesium ions within the chloroplast is among the mechanisms that regulate the photosynthetic reactions. However, a detailed understanding of the dynamics of the movement of these ions is lacking, in part, because traditional methods of measuring these ions inside living cells cannot definitively differentiate between the two ions. We have developed an experimental approach to measure calcium ions in living plants using the light-emitting protein aequorin; a protein that is activated only by calcium. Once the exact calcium concentration is known then fluorescent dyes that are semi-selective for magnesium and calcium can be used to determine the magnesium concentration. With the aid of the instrument acquired through this award, we will be able to make simultaneous measurements of calcium and magnesium concentrations inside the plant chloroplast and also measure the extent of the changes of the concentration of these ions during the transition from light to dark. Thus, this instrument will greatly facilitate our research. Gaining a better understanding of all of the elements that regulate photosynthesis will give agronomists a broader array of tools with which to manipulate and hopefully enhance crop productivity.

2000-03384 Biochemistry of Oilseeds: New Strategies for Improving Oils

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Grant 2001-35318-10186; $180,000, 3 Years

Many plants accumulate seed reserves as oils composed of triacylglycerols. These vegetable oils constitute important sources of food and industrial products with commercial production worth $25 billion worldwide. To a large extent, the properties of different oils depend on the degrees of unsaturation of the fatty acid components and this is a function of desaturase enzymes that introduce double bonds into the fatty acid chains. There is considerable interest among plant breeding and biotechnology companies in producing modified oil crops, which would help to diversify farm economies and to advance sustainable agricultural systems. Conventional and mutation breeding have provided some desirable alterations in the composition of several oilseeds. However, very few changes that require more sophisticated genetic engineering techniques have been possible because the biochemistry and regulation of oilseed triacylglycerol synthesis

are not well understood and because only in the last few years has it been possible to isolate and clone some of the genes involved.

This proposal describes experiments to characterize the biochemistry of these desaturases following their expression in transgenic yeast. The information gained will be used to refine strategies for altering seed oil composition. In one approach, genes that encode palmitic acid desaturases will be transformed into Arabidopsis to explore different options for reducing the saturated fatty acid content of oils. Other desaturase genes will be tested for their ability to direct the synthesis of long-chain polyunsaturated fatty acids in seeds. We expect these approaches to bring about useful modifications in oil composition. Recent examples indicate that understanding and altering the enzymatic machinery of oilseeds will be a key to successful engineering of oil composition.

2000-03353 Metabolic Engineering of Plants for Enhanced Productivity

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Grant 2001-35318-10126; $180,000; 3 Years

Plant productivity and crop yields are governed by the rate and duration of photosynthesis and the partitioning of fixed carbon between sucrose and starch in source leaves as well as the capacity of sink tissues to assimilate sugars and amino acids from source leaves. Although the basic framework of physiological and metabolic processes controlling productivity and yields are well established, the exact molecular details remain largely undefined. In this research project supported by USDA-NRICGP funds, an integrated molecular, biochemical and physiological approach will be employed to study processes that govern source-sink relationships in the ideal genetic system *Arabidopsis* and in a model crop plant rice. In *Arabidopsis*, we will continue ongoing studies to determine the biochemical basis that is responsible for the significant correlation between photosynthetic capacity, plant growth and leaf starch. In rice, a plant which displays an unusual sensitivity to feedback of photosynthesis under moderate environmental conditions, we will evaluate the role of starch metabolism in leaves and in developing seeds in effecting plant productivity and yield. These studies will aid in efforts to increase our understanding of carbon partitioning during plant development and may lead to novel approaches to significantly increase plant productivity to levels not attainable by traditional breeding programs.